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Influence of Light and Nutrients on Atrazine Toxicity to Freshwater Algae

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INFLUENCE OF LIGHT AND NUTRIENTS ON ATRAZINE TOXICITY TO FRESHWATER ALGAE

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In the midwestern United States, the extensive use of atrazine has led to its widespread occurrence in surface waters. Toxic levels of atrazine have been established for many algal species. However, the role of environmental factors in toxicity determinations has not often been considered. Ambient conditions may influence response to exposure, thus altering atrazine's ecological impact.

This study examined the influence of two environmental factors, nutrients and light, on atrazine toxicity to two species of freshwater algae. A split-plot design included three levels of light (5, 100, 500 μmol/m^2/s) and phosphorus (16, 160, 1600 μg/L) with four levels of atrazine per species (0, 1, 50, 200 μg/L for *Ankistrodesmus falcatus*; 0, 200, 500, 1000 μg/L for *Cyclotella menenghiniana*). Algal growth was tracked with a fluorometer over a nine-day period using percent inhibition as a measure of toxicity. Interactions between light and atrazine were significant on all days for *A. falcatus*. Percent inhibition increased with increasing atrazine concentrations at medium and high light levels, while growth was generally minimized by low light. Response of *C. menenghiniana* was more varied and appeared to be more influenced by interactions among atrazine, light and phosphorus. Regardless of light level, highest atrazine concentration was least inhibitory at high phosphorous concentration.

Several factors influence algal response to atrazine exposure, including physiological, structural and community differences. However, this research demonstrated that environmental conditions also play an integral role in determining atrazine toxicity.

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INTRODUCTION

Atrazine, a triazine herbicide, has been used extensively in agricultural regions. Its presence in surface waters in the midwestern United States has been well documented (Thurman et al. 1992, Stamer et al. 1994, Solomon et al. 1996). The levels of atrazine present in streams may differ according to watershed use and seasonal variations in precipitation. First-order streams directly adjacent to agricultural fields may be exposed to high concentrations during storm runoff. This is especially prevalent in the spring as newly applied agrichemicals are washed into streams by frequent and intense rainfall (Thurman et al. 1991). Atrazine levels as high as 691 μg/L have been documented (Langan et al., 1995). This concentration is temporary as the atrazine is diluted or degraded (Solomon et al., 1996); however, because of consistent seasonal use, atrazine may be continuously present in higher order streams, such as the mean concentration of approximately 0.8 μg/L reported for the Platte River in Nebraska throughout much of the year (Nelson et al., 1997).

As the runoff enters an aquatic system, non-target organisms are exposed to potentially toxic pesticide levels. The effect of pesticides on non-target organisms, such as algae, has been the subject of several investigations (Huber, 1993; Hoagland et al., 1996; Solomon et al., 1996). In laboratory studies, atrazine levels as low as 1-5 μg/L affected photosynthesis in some algae (DeNoyelles et al., 1982). At 20 μg/L under field and laboratory conditions, both photosynthesis and community succession were influenced (DeNoyelles et al., 1982). At levels of 300 μg/L and higher, total algal

biomass reductions of 91% have been found (Pratt et al., 1988). In reviews of toxic atrazine levels, Huber (1993) and Solomon et al. (1996), concluded that concentrations <20 μg/L would not be expected to have prolonged effects on organisms in flowing or standing water, regardless if the atrazine addition was chronic or acute. In contrast, Nelson et al. (1997) revealed that the diatom *Craticula cuspidata* was adversely affected by prior exposure to low levels of atrazine. Thus, chronic exposure to low atrazine concentrations is potentially inhibiting, although the inhibition may not be apparent until exposure to higher levels of atrazine occurs.

The level at which pesticides become toxic is correlated to its persistence in the environment, the susceptibility of exposed organisms, and ambient water quality conditions. Despite the widespread occurrence of atrazine in midwestern streams and rivers, its ecological impacts are difficult to predict. This is in part due to varying ambient conditions, including nutrient levels (Gunnarsson et al., 1995; Detenbeck et al., 1996) and light levels (Mayasich et al., 1986; Mayer et al., 1998), which can alter atrazine's effects on algae.

Nutrient and pesticide interaction

Several studies have examined the interaction between nutrients and pesticides. Exposure to pesticides may result in reductions in nutrient uptake by algae (Krieger et al., 1988; Detenbeck et al., 1996). The level of nutrients available to algae also may have an

effect on a pesticide's disappearance rate from the water column (Barreiro and Pratt, 1994). Algae that have excess nutrients available may be better able to tolerate exposure to a toxicant than those with lower nutrient resources. Recovery to control values following herbicide exposure may be higher when nutrient levels are also elevated (Barreiro and Pratt, 1994). Nutrient levels can affect algal sensitivity to pesticides if the nutrients compete with the pesticide for binding sites (Wangberg and Blank, 1990). If the shift in nutrient supply favors species more sensitive to a pesticide, algal biomass could be more suppressed than without nutrient addition (Detenbeck et al., 1996). Tubea et al. (1981) studied combinations of pH and several nutrients with four different pesticides, using two species of algae. Herbicide effects varied with different nutrient and pH levels, indicating the importance of considering these factors when determining ecotoxicity.

Algae also experience indirect effects of pesticide/nutrient interactions. Pesticide additions may reduce predation by inducing zooplankton mortality and earlier emergence of herbivorous insects (Dewey, 1986). Algae would experience dual benefits: increased nutrient availability and decreased grazing pressure (Van Donk et al., 1995). However, Waiser and Robarts (1997) showed that bacterial growth might increase with simultaneous additions of nutrients and pesticide, therefore increasing nutrient competition.

Light and pesticide interaction

Light is essential in algal photosynthesis. Algae are able to photoadapt to the prevailing light conditions (Boston and Hill, 1991). Photoacclimation allows algae to maintain constant net growth efficiency over a broad range of irradiance levels (Falkowski and LaRoche, 1991). This ability may be beneficial in optimizing growth, but may be detrimental in the presence of a pesticide.

Millie et al. (1992) found that algal communities acclimated to higher photon flux densities may be less able to quench excess light energy, resulting in immediate photosynthetic inhibition upon exposure to the herbicide simazine. In algae that were not pre-adapted to specific light levels, atrazine was more toxic under low than high light (Mayer et al., 1998). Inhibition due to atrazine was significantly reduced under mild photoinhibition due to high light intensity (Mayer et al., 1998).

Species-specific responses to light intensity and pesticide concentrations display wide variation. The chlorophyte, *Nannochloris oculata* Droop, was most inhibited by atrazine at high light and temperature, whereas the diatom *Phaeodactylum tricornutum* Bohlin was inhibited at low light regardless of temperature (Mayasich et al., 1986).

The influence of environmental conditions on algal response to pesticide exposure has not often been considered. This study focused on the effects of pesticide exposure relative to two environmental conditions (nutrients and light), which may vary dramatically during a runoff event.

MATERIALS AND METHODS

Algal cultures

Common to freshwater systems and easily cultured, the diatom, *Cyclotella meneghiniana*, and the green alga, *Ankistrodesmus falcatus*, were utilized. *A. falcatus* was obtained from the University of Texas Algal Culture Collection (Austin, TX, USA) and *C. meneghiniana* from the Loras College Freshwater Diatom Culture Collection (Dubuque, IA, USA).

Using 25 x 150 mm culture tubes, both species were cultured for two weeks in 40 mL modified WC medium (with doubled amount of silica, addition of ferric sequestrine, and 160 µg/L phosphorus) prior to initiating experiments. The tubes were maintained at a constant 20°C temperature, 500 µmol/m^2/s on a 12:12 light:dark cycle. This light level has been shown to achieve saturation for freshwater benthic algae without inducing photoinhibition (Hill, 1996).

Experimental design

A split-plot design was used for all experiments, utilizing the following levels of three factors: (1) light levels at approximately 5, 100 and 500 µmol/m^2/s (Hill, 1996); (2) phosphorus concentration (as KH_2PO_4) at 16, 160, and 1600 µg/L (based on unpublished USGS data for agricultural streams in Nebraska); (3) atrazine at 0, 1, 50, and 200 µg/L (Solomon et al., 1996) for *A. falcatus* and 0, 200, 500 and 1000 µg/L for *C. meneghiniana* (Solomon et al., 1996, and a preliminary experiment demonstrating no significant effect

on *C. meneghiniana* for concentrations <200 μg/L). Each treatment combination was replicated twice.

Light

Using cool white fluorescent bulbs, each light level was replicated in two growth chambers for a total of six chambers. Light intensity was varied with different numbers of bulbs. At the highest level, a diffuser was placed over the bulbs and the racks of tubes were placed upon it. The lowest light level was achieved using neutral density plexiglass filters, and checked for spectral distribution with a Li-Cor 1800UW spectroradiometer (LiCor, Lincoln, NE, USA).

Phosphorus

A 50-mg/L stock solution of KH_2PO_4 was prepared according to Lind (1985). Respective amounts of stock solution were added to make three liters of medium for each phosphorus concentration. Initial and final phosphorus concentrations were determined by a modified Lind method for total phosphorus (1985, based on Menzel and Corwin, 1965 and Murphy and Riley, 1962). Final concentrations included the phosphorus content of the algae.

Atrazine

Technical grade atrazine (99% purity), obtained from Chem Service (West Chester, PA, USA), was dissolved in 100% ethanol to make a stock solution of 0.1

mg/mL. The solution was filter sterilized using Millex-GS 0.22 μm filters (Millipore, Bedford, MA, USA). The atrazine stock was then further diluted with filter-sterilized ethanol to create sub-stock solutions. Two-milliliter aliquots were added to autoclaved medium, achieving desired concentrations. Two milliliters of ethanol (0.3%) were added to each control medium. Using sterile technique, 40 mL of the medium, containing the desired combinations of atrazine and phosphorus, was dispensed into previously autoclaved culture tubes.

Samples for determination of initial and final atrazine concentrations were stored at 4°C in acid-washed, hexane-rinsed amber glass bottles. Final concentrations for the highest light level/phosphorus/atrazine concentration for each species were determined. These final solutions were filtered to remove algae using Type A/E glass fiber filters (Pall Gelman, Ann Arbor, MI, USA). Atrazine samples were analyzed at the University of Nebraska Water Sciences Laboratory (Lincoln, NE, USA).

Algal transfer and growth monitoring

Prior growth experiments that tracked algal growth with a fluorometer (Turner Designs, Sunnyvale, CA, USA) were used to determine the algal density required to transfer to fresh medium, and respective amounts of each algal species were transferred into culture tubes.

Fluorometer readings were taken on day 0 (immediately after treatment) and days 1, 3, 5, 7 and 9. Cell doubling per day was always positive before day 9 in prior growth experiments. Cell doublings per day were calculated using the formula: ln F2 - ln F1 / t_2-t_1 (ln 2) where F2 = fluorometer reading at time 2, F1 = fluorometer reading at time 1, t_2 = time 2 and t_1 = time 1 (Guillard, 1973).

Cell counts were also performed on days 0, 1 and 7 using a Sedgewick-Rafter cell (Wildco, Saginaw, MI, USA). Correlation between fluorometer readings and cell counts was determined for each respective atrazine concentration for each species. Biovolumes of twenty-five samples of each species were calculated according to formulas provided by Hillebrand et al. (1999). Measurements were made with a Nikon compound microscope equipped with an ocular micrometer.

Statistical analyses

An ANOVA for each species, each day, was performed using SAS software (SAS Institute, Inc., Cary, NC, USA). Percent inhibition was calculated using the formula: [1-(μi/μc)]100 where μi = growth rate of culture at specific atrazine, phosphorus and light level, μc = growth rate of control (0 μg/L atrazine).

RESULTS

Initial and final atrazine and phosphorus concentrations were determined as shown in Table 1. Interactions between light and atrazine were significant on all days for

the green alga *A. falcatus* (Table 2). Regardless of atrazine concentration, growth was suppressed at low light (5 μmol/m^2/s). At higher light levels, there was more variation in growth among atrazine levels (Figure 1). Atrazine caused greater inhibition at 1 μg/L than 50 or 200 μg/L under low light conditions, while at medium (100 μmol/m^2/s) and high (500 μmol/m^2/s) light levels, inhibition generally increased with increasing atrazine concentrations. Percent inhibition at low light and 1 μg/L atrazine was similar to inhibition at medium and high light at 200 μg/L atrazine (Figure 2).

The diatom *C. menenghiniana* exhibited a much more varied response (Figure 3). Phosphorus treatment was significant on days 1 and 3, while on day 5 there was a phosphorus/light interaction. Atrazine effect was significant at concentrations ≥500 μg/L individually on days 1, 5 and 7, but interacted with light only on day 3. Light effects were individually significant on day 7. None of the factors had significant effects on day 9 (Table 3). At all light levels and highest atrazine concentration, percent inhibition was lowest at the highest phosphorus concentration (Figure 4).

On days 0 and 1, the correlation between fluormeter readings and cell counts had a Pearson's correlation coefficient of <0.48 for both species. By day 7, fluorescence and cell density were highly correlated for both *A. falcatus* (>0.92) and *C. menenghiniana* (>0.84).

DISCUSSION

Ankistrodesmus falcatus

Light levels were more significant than phosphorus levels in interactions with atrazine and subsequent effects on growth of *A. falcatus*. Regardless of atrazine level, growth at low light was minimal. With the growth suppression induced by light levels, atrazine's effect was negligible. Enhanced growth at medium and high light levels appeared to intensify the susceptibility to atrazine; inhibition increased as concentration of atrazine increased. Mayasich et al. (1986) reported that a green alga's positive growth response to increased light and temperature intensified its vulnerability to atrazine, with greatest inhibition at high light and temperature. Mayer et al. (1998) provided contrasting evidence of atrazine being more toxic under light limiting conditions, regardless of temperature.

Phosphorus effects were insignificant for *A. falcatus* except on day 5. Cell doublings per day reached a maximum on day 5 at low and high light (day 7 for medium light). Growth levels generally declined after day 5, which was particularly evident at high light.

Cyclotella meneghiniana

C. meneghiniana's response to changes in light level was more varied and appeared to be influenced more by interactions with other variables. Growth at low light was minimal. *C. meneghiniana* grew at a slower rate than *A. falcatus* (Table 4). The delayed response to changes in light level may be beneficial for diatoms, decreasing their

susceptibility to atrazine. Response to atrazine exposure remains constant and is not intensified by immediate reactions to irradiance changes (Mayasich et al., 1986).

Both species were photoacclimated to 500 μmol/m^2/s prior to atrazine exposure. Pre-acclimated cells may have protective mechanisms that dissipate excess radiant energy induced by atrazine (Mayasich et al., 1986). Millie et al. (1992) suggested that acclimation may mask ecotoxicological response to a herbicide. Acclimation to different photon flux densities could therefore alter the ability to withstand herbicide exposure, particularly those affecting the photosynthetic apparatus. However, due to *C. meneghiniana*'s delayed growth response to irradiance changes, prior acclimation to high light may have been detrimental following subsequent exposure to low light. Percent inhibition was generally elevated at low light.

Cyclotella meneghiniana may be able to utilize heterotrophy, thus reducing its vulnerability to photosynthetic disruption. Heterotrophic behavior in algae has been suggested as an adaptation to limited irradiance levels (Tuchman, 1996) and disruption by photosynthetic inhibitors (Bérard et al., 1999b). Algae able to substitute heterotrophic for autotrophic metabolism would have a competitive advantage for survival in temporarily altered conditions inhibiting photosynthesis.

Phosphorus effects on *C. meneghininana* were significant individually on days 1 and 3, while interacting with light on day 5. Shabana (1987) found that atrazine addition enhanced phosphorus accumulation in some cyanobacteria. Phosphate enrichment

enabled atrazine to be more effective in reducing periphyton carotenoid/chlorophyll a ratios (Detenbeck et al., 1996).

Atrazine concentrations above 200 μg/L were required to generate an effect in *C. meneghiniana*. Diatoms have been shown to be generally more tolerant to pollution (Guasch et al., 1998) and atrazine (DeNoyelles et al., 1982; Gurney and Robinson, 1989; Hoagland et al., 1993; Tang et al., 1997; Bérard et al., 1999b).

Differential toxicity

Differences in optimum growth conditions and physiological/structural processes must be considered when comparing green algae with diatoms. Chlorophytes demonstrate a positive growth reaction to increasing irradiance, more so than diatoms (Mayasich et al., 1986; Hill, 1996). Reliance on autotrophy increases susceptibility to factors affecting photosynthesis, including irradiance fluctuations and photosynthetic inhibitors. Brown and Lean (1995) found that atrazine was highly inhibitory to carbon uptake at 100 μg/L, while phosphate assimilation was unaffected until >10,000 μg/L. Atrazine may also enhance metabolic activity, that is NH_4^+ and PO_4^{-3} uptake, while inhibiting growth, up to a certain concentration (Shabana, 1987). Tang et al. (1998) found that, over a similar time period, atrazine uptake was higher in chlorophytes as compared to diatoms and bioaccumulation of atrazine was significantly correlated with cell size. In this study, however, biovolume of *C. meneghiniana* ($229 \pm 87 \mu m^3$) was less than that of *A. falcatus* ($356 \pm 228 \mu m^3$). King (2000) found that the relationship between

cell size and atrazine toxicity was not significant, although smaller cells were generally more susceptible.

Importance of interactions

The combination of high phosphorus with high or medium light intensity decreased percent inhibition at the highest atrazine concentration in *C. meneghiniana.* Optimum levels of growth-inducing factors may lessen the effects of atrazine for some algal species. The importance of factor interactions has been documented, such as between light and phosphorus limitation (Wehr, 1993), irradiance, nutrients and herbivory (Rosemond, 1993), and structure, physiology and environmental conditions (Boston and Hill, 1991). Thus, atrazine efficacy as it relates to these interactions must be considered.

Identifying a specific factor influencing atrazine toxicity is difficult. Atrazine effects appear to be dependent on an individual species' sensitivity, community competition and environmental factors (Bérard et al., 1999a). Krieger et al. (1988) and Detenbeck et al. (1996) found that atrazine effects were not lessened by increasing nutrient supply, as lowered algal productivity equated with lowered nutrient uptake capacity. However, recovery from pesticide exposure was enhanced by high nutrient levels (Barreiro and Pratt, 1994) and increased temperature (Bérard et al., 1999a). Phosphorus enrichment may also shift community composition to domination by atrazine-susceptible species (Detenbeck et al., 1996). During seasonal decline of

nutrients, algae and sediments may bioaccumulate pesticide due to decreased bacterial utilization of the pesticide as a carbon source (Waiser and Robarts, 1997).

Seasonal changes may influence every aspect of algal response, from community restructuring to individual physiology. Fluctuations in water flow during summer months may mask response to atrazine as algae adapt to changing conditions (Guasch et al., 1997). Atrazine inhibition of small chlorophytes during the "clear water" phase of early summer coincided with heavy zooplankton grazing and high irradiance levels to severely alter algal community composition more than in any other season (Bérard et al., 1999b). Atrazine was more toxic to communities in open stream sites (average irradiance, 1257 $\mu mol/m^2/s$) as opposed to shaded sites (103 $\mu mol/m^2/s$) in summer months. Also, contrasting the same open sites in summer and winter (345 $\mu mol/m^2/s$) resulted in higher toxicity in the summer (Guasch et al., 1997). Caux and Kent (1995) found an atrazine effect at lower concentrations during the fall rather than the spring. Water quality parameters differed significantly between the two seasons, indicating their potential role in toxicity determination.

Standard bioassays generally do not consider variations in experimental conditions, instead focusing on the manipulation of a single factor while other variables are held constant. With this approach, it is not possible to identify interaction between factors. This study has shown that it is important to consider the variability of test conditions on toxicity test results, as variations in light and phosphorus levels had

significant effects on atrazine toxicity to freshwater algae. Complex interactions present in nature cannot be fully duplicated in experimental procedures; however, a factorial design allows for a more realistic representation of these interactions. Understanding the role that each factor plays, in concert with species- and community-level responses, will provide greater insight into the impact of atrazine on aquatic ecosystems.

REFERENCES

Barreiro Lozano R, Pratt JR. 1994. Interaction of toxicants and communities: the role of nutrients. *Environmental Toxicology and Chemistry* 13:361-368.

Bérard A, Leboulanger C, Pelte T. 1999a. Tolerance of *Oscillatoria limnetica* Lemmermann to atrazine in natural phytoplankton populations and in pure culture: influence of season and temperature. *Archives of Environmental Contamination and Toxicology* 37:472-479.

Bérard A, Pelte T, Druart JC. 1999b. Seasonal variations in the sensitivity of Lake Geneva phytoplankton community structure to atrazine. *Archiv für Hydrobiologie* 145:277-295.

Boston HL, Hill WR. 1991. Photosynthesis-light relations of stream periphyton communities. *Limnology and Oceanography* 36:644-656.

Brown LS, Lean DRS. 1995. Toxicity of selected pesticides to lake phytoplankton measured using photosynthetic inhibition compared to maximal uptake rates of phosphate and ammonium. *Environmental Toxicology and Chemistry* 14:93-98.

Caux PY, Kent RA. 1995. Towards the development of a site-specific water quality objective for atrazine in the Yamaska River, Quebec, for the protection of aquatic life. *Water Quality Research Journal of Canada* 30:157-178.

DeNoyelles F, Kettle WD, Sinn DE. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. *Ecology* 63:1285-1293.

Detenbeck N, Hermanutz R, Allen K, Swift M. 1996. Fate and effects of the herbicide atrazine in flow-through wetland mesocosms. *Environmental Chemistry and Toxicology* 15:937-946.

Dewey S. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. *Ecology* 67:148-162.

Falkowski PG, LaRoche J. 1991. Acclimation to spectral irradiance in algae. *Journal of Phycology* 27:8-14.

Guasch H, Muñoz I, Rosés N, Sabater S. 1997. Changes in atrazine toxicity throughout succession of stream periphyton communities. *Journal of Applied Phycology* 9:137-146.

Guasch H, Ivorra N, Lehmann V, Paulsson M, Real M, Sabater S. 1998. Community composition and sensitivity of periphyton to atrazine in flowing waters: the role of environmental factors. *Journal of Applied Phycology* 10:203-213.

Guillard RRL. 1973. Division rates. In J.R. Stein ed., *Handbook of Phycological Methods: Culture Methods and Growth Measurements*. Cambridge University Press, London, UK, pp.289-311.

Gunnarsson J, Broman D, Jonsson P, Olsson M, Rosenberg R. 1995. Interactions between eutrophication and contaminants: towards a new research concept for the European aquatic environment. *Ambio* 24:383-385.

Gurney SE, Robinson GGC. 1989. The influence of two triazine herbicides on the productivity, biomass and community composition of freshwater marsh periphyton. *Aquatic Botany* 36:1-22.

Hill W. 1996. Effects of light. In R.J. Stevenson, M.L. Bothwell, and R.L. Lowe, eds., *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, Inc. San Diego, CA, USA, pp.121-148.

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* 35:403-424.

Hoagland KD, Drenner RW, Smith JD, Cross DR. 1993. Freshwater community responses to mixtures of agricultural pesticides: effects of atrazine and bifenthrin. *Environmental Toxicology and Chemistry* 12:627-637.

Hoagland KD, Carder JP, Spawn RL. 1996. Effects of organic toxic substances. In R.J. Stevenson, M.L. Bothwell, and R.L. Lowe, eds., *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, Inc. San Diego, CA, USA, pp.469-496.

Huber W. 1993. Ecotoxicological relevance of atrazine in aquatic systems. *Environmental Toxicology and Chemistry* 12:1865-1881.

King C. 2000. Selective atrazine toxicity in freshwater algae. MS Thesis. University of Nebraska, Lincoln, NE, USA.

Krieger KA, Baker DB and Kramer JW. 1988. Effects of herbicides on stream aufwuchs productivity and nutrient uptake. *Archives of Environmental Contamination and Toxicology* 17:299-306.

Langan MM, Hoagland KD, Everson AR. 1995. Pesticide levels in storm runoff from agricultural stream sites with different riparian buffer strips. *Proceedings: Platte River Basin Ecosystem Symposium*, Kearney, NE, USA. (Feb. 28-Mar. 1, 1995) pp.223-233.

Lind OT. 1985. *Handbook of common methods in limnology*. Second edition. Kendall/Hunt Publishing Company, Dubuque, Iowa, USA. pp.77-83.

Mayasich JM, Karlander EP, Terlizzi, Jr DE. 1986. Growth responses of *Nannochloris oculata* Droop and *Phaeodactylum tricornutum* Bohlin to the herbicide atrazine as influenced by light intensity and temperature. *Aquatic Toxicology* 8:175-184.

Mayer P, Frickmann J, Christensen ER, Nyholm N. 1998. Influence of growth conditions on the results obtained in algal toxicity tests. *Environmental Toxicology and Chemistry* 17:1091-1098.

Menzel DW, Corwin N. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. *Limnology and Oceanography* 10:280-282.

Millie DF, Hersh CM, Dionigi CP. 1992. Simazine-induced inhibition in photoacclimated populations of *Anabaena circinalis* (cyanophyta). *Journal of Phycology* 28:19-26.

Murphy J, Riley J. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.

Nelson KJ, Hoagland KD, Siegfried BD. 1999. Chronic effects of atrazine on tolerance of a benthic diatom. *Environmental Toxicology and Chemistry* 18:1038-1045.

Pratt JR, Bowers NJ, Niederlehner BR, Cairns, Jr J. 1988. Effects of atrazine on freshwater microbial communities. *Archives of Environmental Contamination and Toxicology* 17:449-457.

Rosemond AD. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. *Oecologia* 94:585-594.

Shabana EF. 1987. Use of batch assays to assess the toxicity of atrazine to some selected cyanobacteria II. Effect of atrazine on heterocyst frequency, nitrogen and phosphorus metabolism of four heterocystous cyanobacteria. *Journal of Basic Microbiology* 27:215-223.

Solomon KR, Baker DB, Richards R, Dixon KR, Klaine SJ, LaPoint TW, Kendall RJ, Weisskopf CP, Giddings JM, Greisy JP, Hall, Jr LW, Williams WM. 1996. Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry* 15:31-76.

Stamer JK, Swanson RB, Jordan PR. 1994. Atrazine in spring runoff as related to environmental setting in Nebraska, 1992. *Water Resources Bulletin* 30:823-831.

Tang J, Hoagland KD, Siegfried BD. 1997. Differential toxicity of atrazine to selected freshwater algae. *Bulletin of Environmental Contamination and Toxicology* 59:631-637.

_____. 1998. Uptake and bioconcentration of atrazine by selected freshwater algae. *Environmental Toxicology and Chemistry* 17:1085-1090.

Thurman EM, Goolsby DA, Meyer MT, Kolpin DW. 1991. Herbicides in surface waters of the midwestern United States: the effect of spring flush. *Environmental Science and Technology* 25:1794-1796.

Thurman EM, Goolsby DA, Meyer MT, Mills MS, Pomes ML, Kolpin DW. 1992. A reconnaissance study of herbicides and their metabolites in surface water of the midwestern United States using immunoassay and gas chromatography/mass spectrometry. *Environmental Science and Technology* 26:2440-2447.

Tubea B, Hawxby K, Mehta R. 1981. The effects of nutrient, pH and herbicide levels on algal growth. *Hydrobiologia* 79:221-227.

Tuchman NC. 1996. The role of heterotrophy in algae. In R.J. Stevenson, M.L. Bothwell, and R.L. Lowe, eds., *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, Inc. San Diego, CA, USA pp.299-319.

Van Donk E, Prins H, Voogd HM, Crum SJH, Brock TCM. 1995. Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms I. Responses of plankton and zooplanktivorous insects. *Archiv für Hydrobiologie* 133:417-439.

Waiser M, Robarts R. 1997. Impacts of an herbicide and fertilizers on the microbial community of a saline prairie lake. *Canadian Journal of Fisheries and Aquatic Sciences* 54:320-329.

Wangberg S, Blank H. 1990. Arsenate sensitivity in marine periphyton communities established under various nutrient regimes. *Journal of Experimental Marine Biology and Ecology* 139:119-134.

Wehr JD. 1993. Effects of experimental manipulations of light and phosphorus supply on competition among picoplankton and nanoplankton in an oligotrophic lake. *Canadian Journal of Fisheries and Aquatic Sciences* 50:936-945.

FIGURES

Figure 1. Growth rates (cell doublings per day) of *Ankistrodesmus falcatus* at three different light intensities and three phosphorus concentrations (Error bars: ± SEM). Atrazine concentrations: (—●—) 0 µg/L, (··○··) 1 µg/L, (—▼—) 50 µg/L, (—▽··) 200 µg/L.

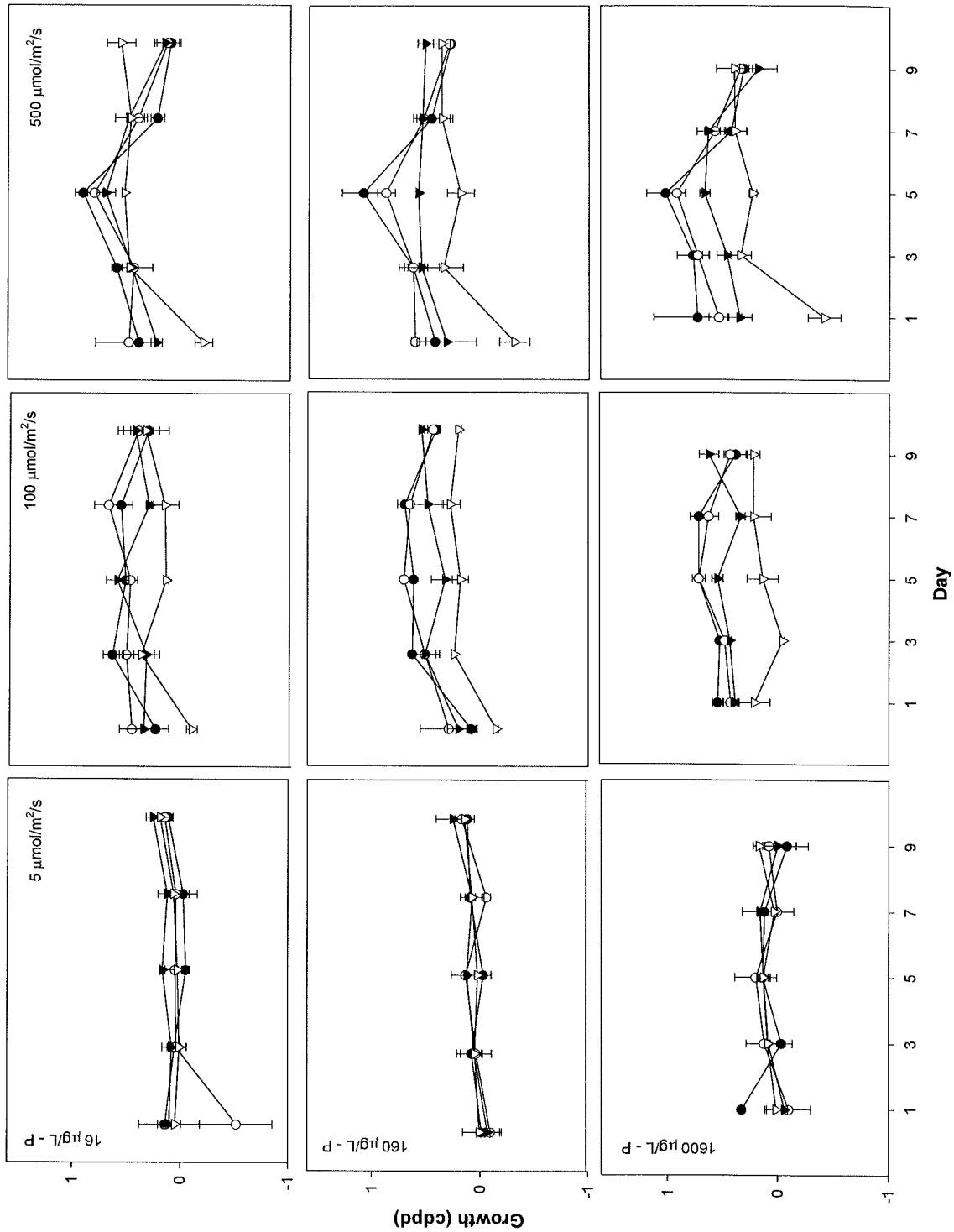

5 µmol/m²/s
100 µmol/m²/s
500 µmol/m²/s
16 µg/L - P
160 µg/L - P
1600 µg/L - P
Growth (cdpd)
Day
1
3
5
7
9
0
-1

Figure 2. Percent inhibition for *Ankistrodesmus falcatus* (relative to controls) according to light level and phosphorus level (—●—) 16 μg/L, (··○··) 160 μg/L, (—▼—) 1600 μg/L.

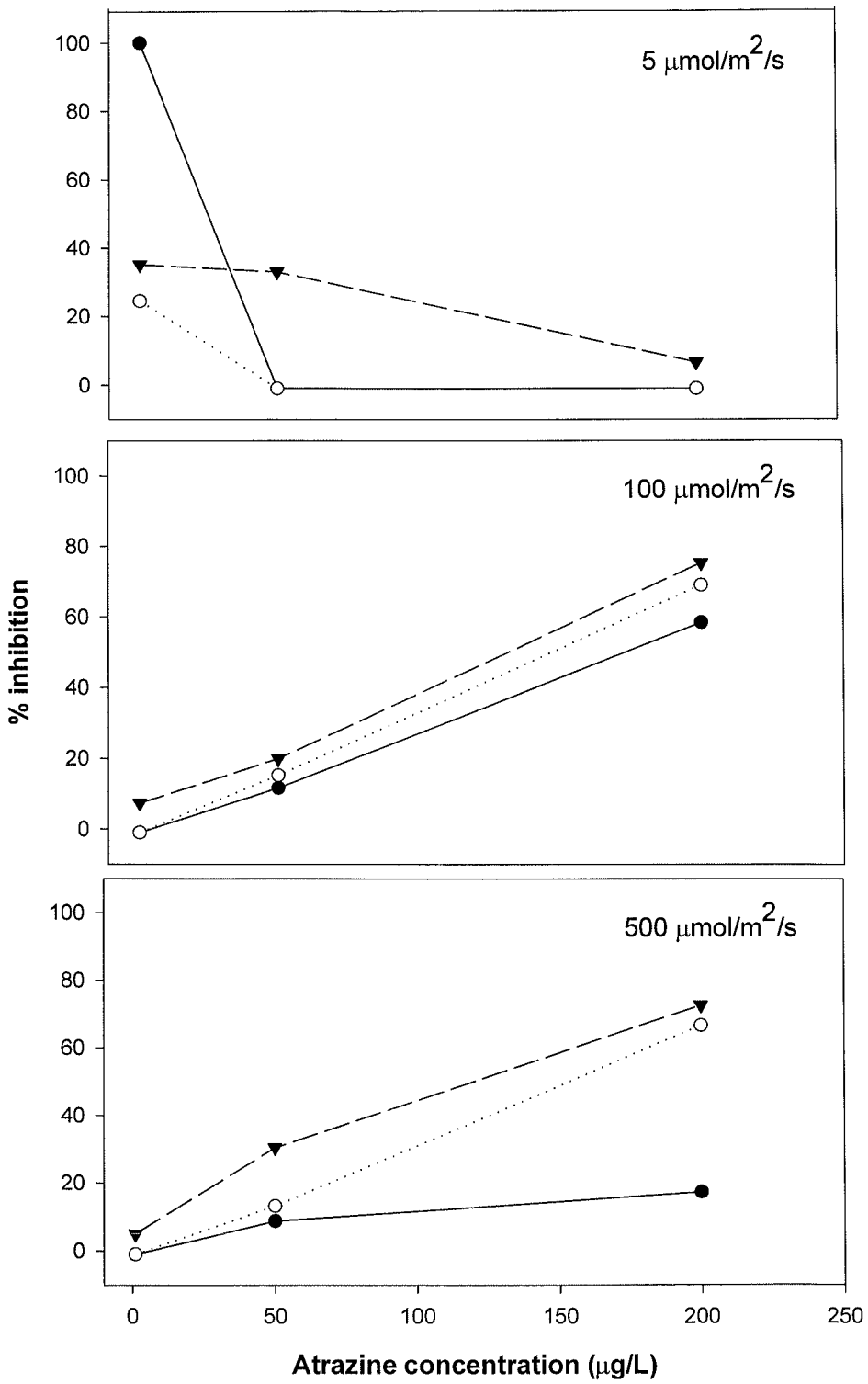

5 μmol/m2/s
100 μmol/m2/s
500 μmol/m2/s
% inhibition
Atrazine concentration (μg/L)
0
20
40
60
80
100
0
50
100
150
200
250

Figure 3. Growth rates (cell doublings per day) of *Cyclotella meneghiniana* at three different light intensities and three phosphorus concentrations (Error bars: ± SEM). Atrazine concentrations: (—●—) 0 µg/L, (··○··) 200 µg/L, (—▼—) 500 µg/L, (—▽··) 1000 µg/L.

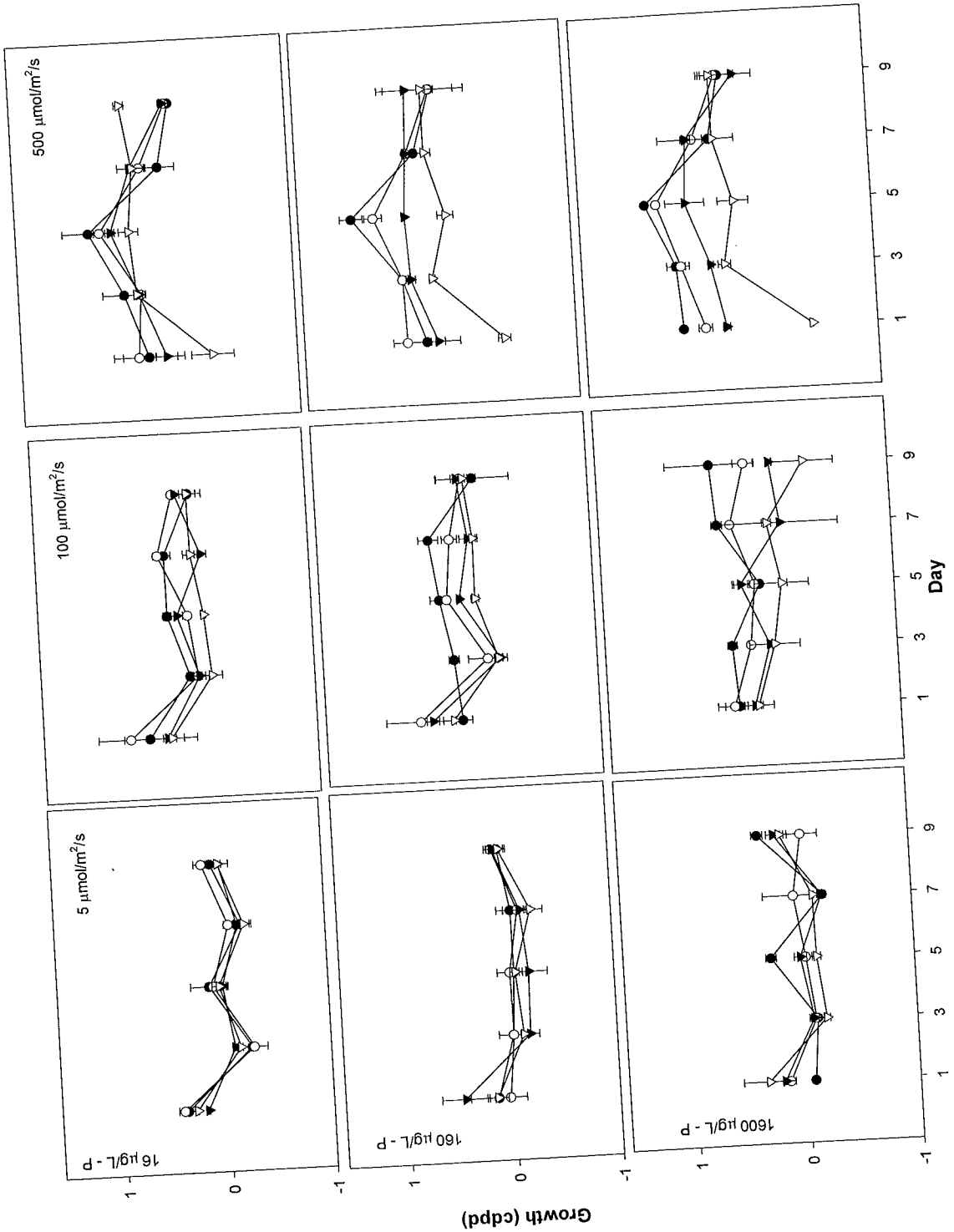
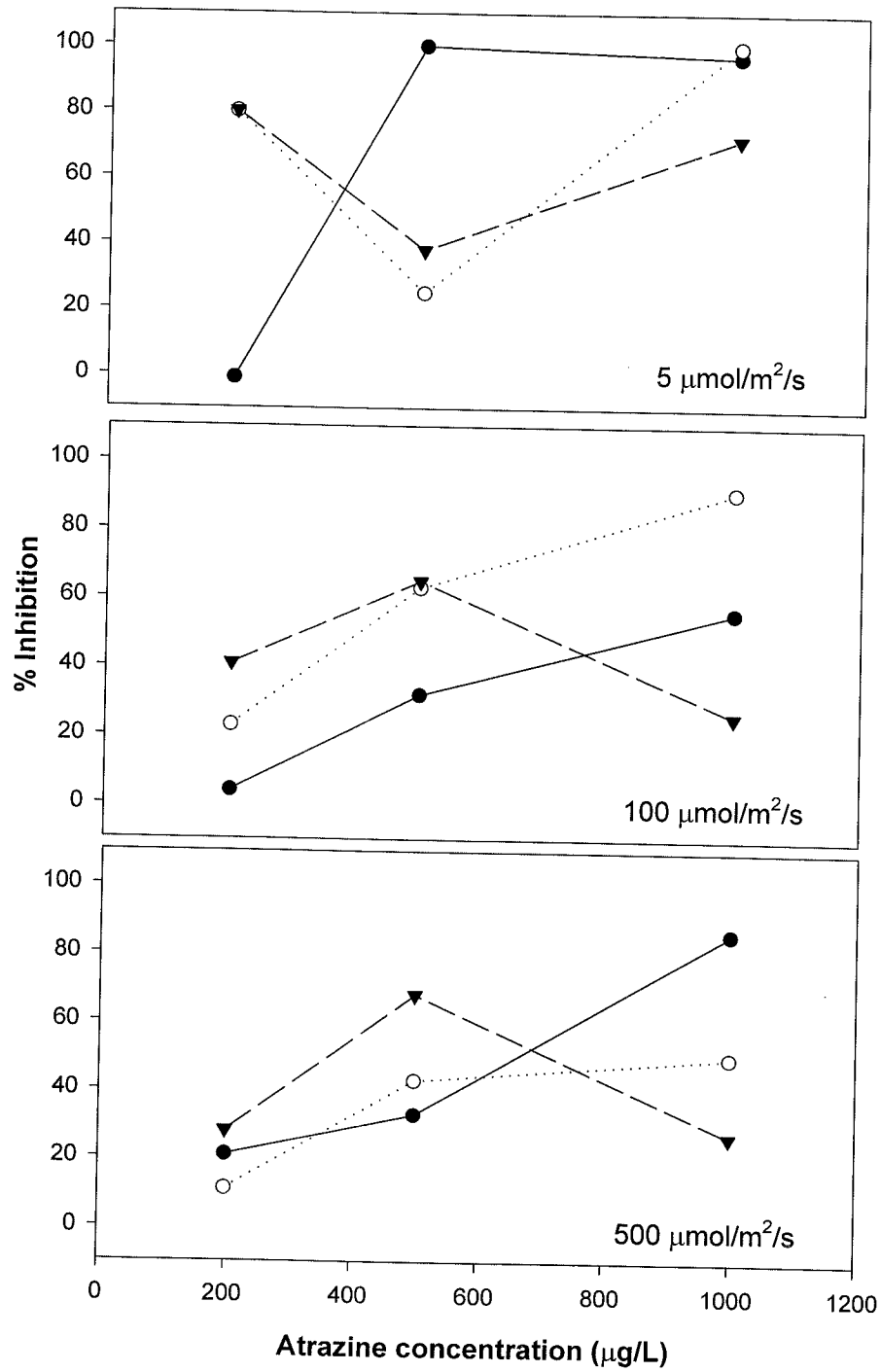

5 µmol/m²/s
100 µmol/m²/s
500 µmol/m²/s
16 µg/L - P
160 µg/L - P
1600 µg/L - P
Growth (cdpd)
Day
1
0
-1
1
3
5
7
9

Figure 4. Percent inhibition for *Cyclotella meneghiniana* (relative to controls) according to light level and phosphorus level (—●—) 16 μg/L, (··○··) 160 μg/L , (—▼—) 1600 μg/L.


100
80
60
40
20
0
5 μmol/m²/s
100
80
60
40
20
0
% Inhibition
100 μmol/m²/s
100
80
60
40
20
0
500 μmol/m²/s
0
200
400
600
800
1000
1200
Atrazine concentration (μg/L)

TABLES

Table 1. Initial and final concentrations (μg/L) of atrazine and phosphorus. Nominal concentrations in parentheses. Final concentrations of atrazine determined from highest light level/atrazine/phosphorus concentration for each species.

		Initial concentration	Final concentration
Atrazine	(1)	1.8	
	(50)	75.6	
	(200)	210.0	
A. falcatus	(200)		157.5
C. meneghiniana	(1000)		752.5
Phosphorus	(16)	26.5	
	(160)	169.0	
	(1600)	1671.6	
A. falcatus	(16)		13.1-69.6
	(160)		150.2-209.4
	(1600)		690.6-1042.8
C. meneghiniana	(16)		23.9-64.2
	(160)		101.8-292.7
	(1600)		432.5-733.7

Table 2. ANOVA summary of significant interactions and, in the absence of an interaction, the significant individual effects for *Ankistrodesmus falcatus* for days 1, 3, 5, 7 and 9; NS = not significant ($p < 0.05$).

	Interaction	p-value	Individual effect
Day 1	Light x atrazine	0.001	NS
Day 3	Light x atrazine	0.020	NS
Day 5	Light x atrazine	0.001	NS
	Phosphorus x atrazine	0.036	
Day 7	Light x atrazine	0.001	NS
Day 9	Light x atrazine	0.014	NS

Table 3. ANOVA summary of significant interactions and, in the absence of an interaction, the significant individual effects for *Cyclotella meneghiniana* for days 1, 3, 5, 7 and 9; NS = not significant ($p < 0.05$).

	Interaction	p-value	Individual effect	p-value
Day 1	NS		Atrazine Phosphorus	0.015 0.033
Day 3	Light x atrazine	0.010	Phosphorus	0.013
Day 5	Light x phosphorus	0.010	Atrazine	0.001
Day 7	NS		Atrazine Light	0.001 0.031
Day 9	NS		NS	

Table 4. Comparison of average growth rates (cell doublings per day) according to light level (μmol/m^2/s) for *Ankistrodesmus falcatus* and *Cyclotella meneghiniana*.

	5	100	500
A. falcatus	0.061	0.403	0.450
C. meneghiniana	0.017	0.256	0.260